THE INFLUENCE OF PHENOBARBITAL ON LEARNING BEHAVIOR IN ALBINO RATS

by

WILLIAM THOMAS WRIGHT

B. S., Southwestern College, 1948

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Education and Psychology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1949

TABLE OF CONTENTS

INTRODUCTION

At the present time the general, if not promiscuous, use of barbiturates is receiving much attention. For the past 20 years the barbiturates have been increasingly used in the medical and psychiatric professions. At first, use of the drug was confined to hospitalized patients; however, in recent years the barbiturates have been increasingly used in the treatment of out-patients. Perhaps the most widely used barbiturate compound is phenobarbital, which is usually administered in tablet form or in its alcohol soluble elixer. This drug is prescribed for both adults and children. The anesthetic, hypnotic and depressant properties of these compounds are of proven value in medicine. But much usage is possibly outside medical practice. Prescriptions have probably been refilled many times without medical consultation.

Much work has been done concerning the effects of this drug on the physiological activity of both man and animals, but nothing has been done to determine its effect on learning. This fact led the investigator to undertake a study of its effect upon learning, first under food motivation and second under stress or punishment, by using rats and not humans as subjects. Rats were chosen both for convenience and in order to control a number of variables not easily controlled with humans. In using animals as subjects any application of generalizations to human behavior or to human experience becomes a mere inference. In this experiment, however, objective observation was used and no

inference was made concerning application to humans.

Materials and Methods

The Maze

The maze used in this experiment was designed by Dr. O. W. Alm of the Department of Education and Psychology at Kansas State College. In this maze the animals are to a large degree isolated from distracting outside stimuli. This is accomplished by enclosing the maze in black drapes. The errors and time scores of running for each animal are observed in an overhanging mirror which can be seen through a small square window in the drape. The alleys are uniformly lighted. While using the maze most of the retracing of the alleys is prevented by closing silent doors after the animals have moved on out of sight. Tracing other animals and stops for traces of animals previously run are almost completely eliminated in this maze due to the fact that the alleys are lifted and ventilated while the black oilcloth floor is being moved to a new position after each run.

The simplicity of this maze can be observed in Fig. 2; however, Alm's previous experiments have proven this maze to be very difficult for the animals to learn. Alm found that, with a reasonably certain criterion of learning (90 per cent perfect runs out of 20 trials), the median number of trials required by 61 mature male rats to reach this criterion was 85. On the

basis of previous experiments Alm found this maze to show a high reliability on error scores and time scores. The coefficients of reliability for the total scores were +.92 and +.93, respectively (1).

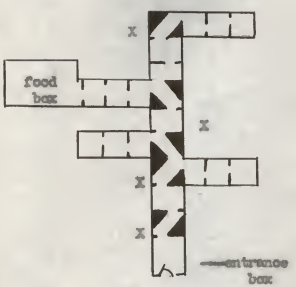


Fig. 1. Diagram to illustrate alley plan for the maze. X's indicate positions of silent doors.

The Stress Box

This apparatus, designed by Alm, is rectangular in shape with a glass front through which the activity of the animal can be observed. The floor of the box consists of a series of electric grids. These grids are wired to a two-way switch mounted on the control panel. By means of this switch current is sent to each half of the floor alternately. The source of current is

a transformer contained in the control box which has an output of 9,000 volts and 1.8 M.A. This amperage is reduced by means of resistors contained in the control box to .2 M.A. and was constantly maintained throughout the experiment as indicated by a M.A. meter on the control panel on the apparatus. The floor plan of this apparatus can be found in Fig. 2. This apparatus is used to condition a response in animals, using electric shock as the adequate stimulus and a buzzer attached to the top of the box as the neutral or conditioned stimulus.

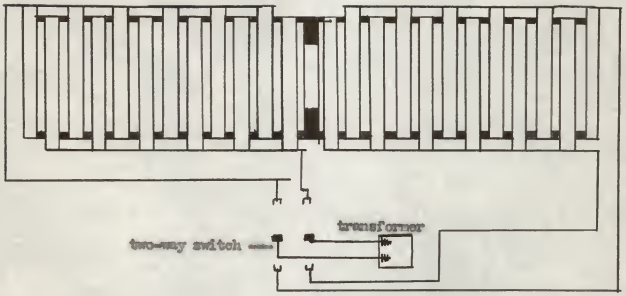


Fig. 2. Diagram illustrating the floor of the stress box and the electric circuit.

Animals Used

The animals used were all male albino rats 55 days old

upon arrival and having a mean weight of 58 grams. At the beginning of the first experiment the animals were 50 days old and after all experiments were completed they were 105 days old.

Handling and Training

The animals were fed a balanced diet of ground feed. Each feeding was supplemented with a small quantity of sunflower seed until the commencement of running the maze, after which sunflower seeds were given only as a reward for running the maze. Each animal was fed once every 24 hours on the completion of his performance for the day.

The animals were divided into two groups of 20 each. They were paired as evenly as possible according to weight and distributed into their groups in order that the difference between the mean weight of the groups might be as small as possible. The group selected to be the experimental group was put on .075 grain of phenobarbital in syrup which was administered orally to each individual at 9 a.m. each day. To equalize handling and diet between the groups, each individual of the control group received an equal quantity of simple syrup. After the animals had been on this dosage for three days, preliminary training was begun.

Both groups received uniform preliminary training in the maze. The first two days five animals were placed in the maze at a time and given three trials to explore the maze. On the

third day following the completion of preliminary training individual trials were started, and the time and error scores were observed and recorded for the first experiment. Further description of training will be given in the discussion under Experimental Data.

Dosage Determination

The determination of the dosage of phenobarbital to be used and the method of administration required study and preliminary experimentation. The method of administration presented the first problem since phenobarbital is soluble only in ether and alcohol. Obviously these solvents could not be used due to their physiological and behavioral effects. For this reason the drug was suspended in a solution of simple syrup.

In order to determine the dosage to be used an experimentation was begun on the basis of .7 grain, which A. L. Tatum found caused complete anesthesia when injected intra-peritoneally in white rats (2). Five animals were selected and administered diminishing dosages of the drug, starting with 0.7 grain in the first animal. The following results were obtained.

Animal	Dosage	Result
1	0.7 grain	Death after 8 hours
2	0.4 grain	Diarrhea and stupor after 8 hours, lasting 16 hours and death after 10 days
3	0.2 grain	Stupor lasting 5 hours and swelling about the eyes
4	0.066 grain	Sluggishness and slight swelling about the eyes
5	0.033 grain	No symptoms seen
6	0.075 grain	Slight swelling about the eyes and greginess after 3 hours

Following this exploratory dosing the experimental group was put on a dosage of .033 grain for a period lasting three days, and since no disabling symptoms were seen the dosage was increased to .066 grain for a period of three days. No symptoms were observed. The dosage was then stepped up to .075 grain, and this dosage was used throughout the experiments because it did not seem to decrease motivation or cause physical disability. The results of this preliminary experimentation seem to indicate that the animals built up a certain degree of tolerance to the drug since a dosage as high as .066 grain caused symptoms of disability in the preliminary experiment and did not when the dosage was later built up to .075 grain.

EXPERIMENTAL DATA

Experiment I

The enclosed maze was used in this experiment. The experimental group was on .075 grain of phenobarbital per rat each day

throughout this experiment. Each rat in both the experimental group and the control group was run five trials per day with an intervening period of from one to two minutes between trials. This procedure continued for 10 days until each rat had run a total of 50 trials. Throughout the investigation the rats were run in the afternoon between the hours of one and five o'clock. Each day the experimental group was run first, and the control group was run last. In order to maintain equal food motivation between the individual animals, each animal was fed immediately after the completion of his last run for the day. The individual animals of each group were run at approximately the same time each day. Error scores and time scores were observed and recorded for each individual rat. The time scores represent the time required for the rat to go from the entrance of the maze to the food box. Time was recorded in seconds. Two errors were counted if the animal entered a blind alley far enough to get his body into at least one segment, and two errors were counted for each segment he passed through in the blind alley. Errors were also counted two-to-the segment if any of the segments were retraced. Some retracing was prevented by closing silent doors behind the animal as soon as it had passed out of sight of the door.

The total number of errors and total time for the 50 runs was recorded on each individual, and these scores were totaled to find the total errors and total time for each group made on the 50 runs. The mean scores and mean time for each group were

then calculated and compared. The results are presented in Tables 1 and 2.

Table 1. Means for the experimental and control groups for time and errors (50 trials).

	Time (sec.)	Errors
Control group	412.15	239.96
Experimental group	435.60	327.40

See appendix for raw scores.

Table 2. Group differences for time and error scores (50 trials).

	Time (sec.)	Errors
Difference between the means	23.45	87.45
Standard error of the difference between the means	35.12	30.27
Significance ratio (t)	.66	2.89
Level of confidence	.50	.01

See appendix for formulae.

A comparison of the mean total error scores for the groups (Table 2) showed a statistically significant difference. The mean for the experimental group was 87.45 higher than the mean for the controls, with a significant ratio of 2.89. This indicates that the drugged animal's performance was interfered

with by the drug. The difference of 23.43 between the mean total time scores seems to indicate considerably better performance for the control group, but the significance ratio was only .66 which means the difference in the means was not statistically significant. This seems to suggest that there was no breakdown in motivation or physical disability due to the drug. The data indicate that due to higher error scores the experimental group ran a greater distance than the controls and at a faster rate since the difference in the means for time scores was not statistically significant.

Experiment II

This experiment is in reality a continuation of Experiment I. The tabulations derived from the first experiment indicate that rats receiving phenobarbital definitely made higher error scores than the control animals on running the maze. Since the data in Experiment I seem to indicate that high error scores in the experimental group were not due to inhibited motivation or physical disability, one might infer that high scores were a result of interference with learning. Such an inference may not be justified, however. Experiment I was not structured to yield such information. It merely indicated that the drugged rats did not perform as efficiently as the non-drugged animals.

Experiment II was set up in an attempt to determine first, whether or not high error scores in the experimental group were

due to an interference with learning, and second, the effect of the drug on the retention of learned activity (in the maze).

In preparation for this experiment the experimental group was taken off the drug immediately after Experiment I had been concluded. Both groups were then put on a 10 day vacation interval. At the end of this interval, both groups were rerun for a total of 25 trials at the rate of five per day in the enclosed maze used in Experiment I. The error and time scores were again observed and recorded. The first 10 runs were considered as a "warm up" period. The last 15 runs of the total 25 were tabulated. Data from these tabulations can be found in Table 4. These data show that there was no statistically significant difference between the means for the groups on error scores. The difference between the means was 8.95, the experimental group being the higher. The significance ratio was 1.36, which does not meet the standard for significance. The difference between the means of the groups on time scores was 11.40 seconds with a significance ratio of 2.24 which almost meets the standard for significance.

Data were then calculated on the last 15 runs of the total 50 made just prior to vacation in Experiment I. The data are presented in Table 3. When the difference between the two groups in mean error scores made prior to vacation was compared to the difference between the mean error scores of the two groups following the vacation, it was found that the experimental group had made 40 per cent less errors following vacation than they

had made prior to vacation while the control group had made 10 per cent less errors following vacation than they had made prior to vacation.

Table 3. Group differences and statistical evaluation (last 15 runs prior to vacation).

	Time (sec.)	Errors
Mean for experimental group	95.25	63.95
Mean for control group	78.75	32.65
Difference between the means	16.50	31.30
Standard error of the difference between the means	6.34	8.56
Significance ratio (t)	2.60	3.66
Level of confidence	0.02	0.001

See appendix for formulae and raw scores.

Table 4. Group difference and statistical evaluation (last 15 following vacation).

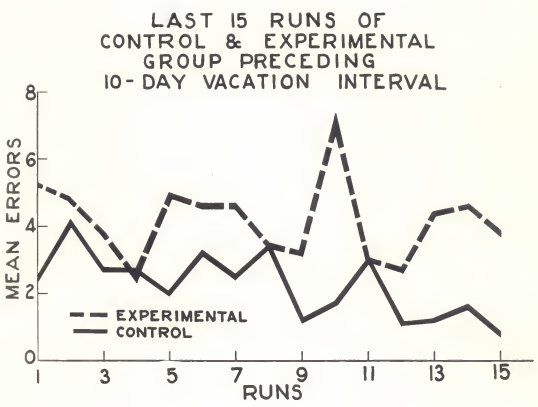
	Time (sec.)	Errors
Mean for experimental group	73.35	38.35
Mean for control group	61.95	29.40
Difference between the means	11.4	8.95
Standard error of the difference between the means	5.08	6.54
Significance ratio (t)	2.24	1.36
Level of confidence	0.05	0.20

See appendix for formulae and raw scores.

After these tabulations were made, a graph (Fig. 3) was plotted to show the mean error scores made by the groups on the last 15 runs previous to vacation and the mean error scores made on the first 15 runs following vacation. This graph indicates that on the first trial following vacation the control group made more errors than they had averaged on any one of the last four runs preceding vacation, while the experimental group made less errors on the first trial following vacation than they ever had made in any trial previous to vacation. In other words, performance of the controls showed the usual loss resulting from vacation, but the experimental animals improved. The results of this experiment seem to indicate that phenobarbital may not have blocked learning in Experiment I but in some way interfered with the efficiency of the rats' performance. It appears that while under the influence of the drug the experimental animals were learning the maze even though they were making high error scores. This experiment also seems to indicate that the drugged animals retained their learned behavior about as well as the control animals. Further comment will be made in regard to retention in the section on discussion.

Experiment III

The purpose of this experiment was to determine the effect of phenobarbital on conditioning under stress, using avoidance of punishment as motivation. The stress box was used to pro-



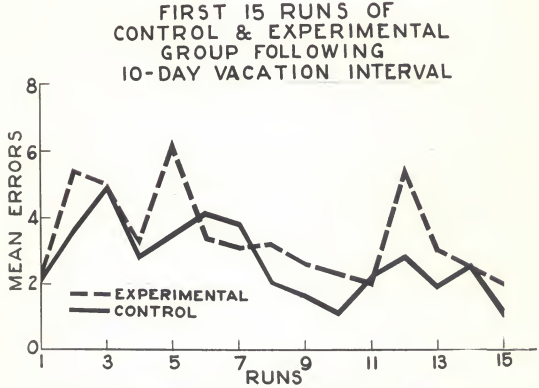


Fig. 3. Mean error scores made by control and experimental groups during maze performance.

vide these experimental conditions. The box provided for the adequate stimulus an electric shock and for the neutral stimulus the sound of a buzzer which was attached to the box.

Throughout the experiment the procedure was as follows: the experimental group was first put on .075 grain of phenobarbital for each rat. This was done three days prior to the start of this experiment. In using the stress box, a rat was put into one end of the box, the buzzer was sounded, and the rat was given 20 seconds to run to the opposite end of the box which was "safe". If it remained longer than 20 seconds it received an electric shock of 0.2 M.A. This procedure was continued alternately from one end of the box to the other for 10 trials at the rate of one every 30 seconds. Each animal in both groups was given a total of 100 trials, or stimulations by the buzzer and possible exposure at the rate of 10 per day.

The stress box provided an extremely upsetting situation to the rats due to the fact that they had to learn to return to the end of the box in which they had just previously received an electric shock in order to escape another shock.

The scores recorded in this experiment fall into two major catagories, learning scores and visceral response scores. Two types of learning scores were recorded. These were signal run scores which consisted of the number of times the rat avoided the electric shock by running to the other end of the box when the buzzer was sounded, and exposure scores, which were the number of times the rat delayed running until after it received

the electric shock. Shock due to retracing was also included in this score. Some of the animals would flash back into the electrified end of the box before the current could be turned off and thus received an exposure score. It was also necessary to cut the current after the animal had passed into the safe end due to a small degree of inductance over the whole floor when the current was on at either end. Visceral response scores consisted of the number of defecation pellets for each rat and the number of urinations made while the animal was under experimental conditions. Defecation pellets were counted for each animal after every 10 exposures. The pellets were caught on a cardboard which was placed under the floor of the stress box. This cardboard was changed for each rat. The number of urinations were also indicated on this cardboard. This method was accurate for defecation pellet counts; however, it is doubtful if it was an accurate method of counting urinations. The inclusion of data derived from this method of counting urinations may be justified by the fact that the same method was used for both groups, and differences thus observed could be significant. Weight loss was also recorded for each group after the experiment had been terminated. Tables 5, 6, 7 and 8 show the resulting data of this experiment.

Table 5. Learning score means for both groups (100 signals).

	Signal runs	Exposures
Control group	12.9	87.70
Experimental group	29.30	71.50

See appendix for raw scores.

Table 6. Group differences on learning scores (100 signals).

	Signal runs	Exposures
Difference between the means	16.40	16.20
Standard error of the difference between the means	3.42	3.47
Significance ratio (t)	4.80	4.64
Level of confidence	.001	.001

See appendix for formulae.

These data indicate that the experimental group made significantly higher signal run scores than the control group. The significance ratio was 4.80. The control group made significantly higher exposure scores than the experimental group; the significance ratio was 4.64. These data indicate that the drugged animals performed more efficiently under stress than the controls.

Table 7. The means of visceral scores (100 signals).

	Defecations	Urinations
Control group	36.25	11.65
Experimental group	16.30	9.40

See appendix for raw scores.

Table 8. Group difference on visceral scores (100 signals).

	Defecations	Urinations
Difference between the means	19.95	2.25
Standard error of the difference between the means	1.92	0.07
Significance ratio (t)	10.05	3.21
Level of confidence	.001	.01

See appendix for formulae.

The control group made significantly higher defecation scores than the experimental group. The control group made significantly higher urination scores than the experimental group. These data indicate that in the drugged animals, visceral responses accompanying conditions of stress were much less likely to occur, whereas the animals not under the influence of the drug made profuse visceral reactions to the stressing conditions.

The reduction in visceral reaction in drugged animals under stress may be due to a lessening in the irritability of the nervous system or an increase in synaptic resistance.

DISCUSSION AND EVALUATION

Dosage Determination

Although each rat in the experimental group was administered 0.075 grain of phenobarbital, they were not receiving the same dosage per-kilogram-body weight, nor were they under the influence of the drug for the same length of time before running. The time interval between the running of the first experimental rat and the last was from one to one and a half hours. The time interval between the administration of the drug to the first and last member of the experimental group was from three to five minutes. These variables however were not of significance in these experiments. The experiments in this study were not planned to determine the effect of the quantity of the drug received by each rat on learning, but to determine whether or not there would be a difference between the drugged and non-drugged animals.

Experiment I

The significant finding in this experiment, that high error scores in the experimental group might not be due to physical disability or interference with food motivation due to the drug, was based upon observations made in this experiment and the preliminary experiment on dosage determination. In these experi-

ments it was observed that the drugged and non-drugged animals consumed their food rations with equal rapidity. The absence of poor food motivation and of physical disability were further indicated by comparing the mean rate of speed for each group. The mean speed rate for the control group was 1.64 maze segments per second. The mean speed rate for the experimental group was 1.78 maze segments per second. The difference between these means was 0.14. Even though the experimental group received higher error scores than the controls in maze running, it was shown that they ran at a more rapid rate which makes it appear that the experimental rats were not suffering from physical disability or poor motivation but were still unable to reduce their error scores.

Experiment II

The original purpose of this experiment was an effort to determine whether or not phenobarbital would have a permanent effect on the learning efficiency in white rats. The data derived from Experiment I and this experiment did not indicate that permanent damage was done by phenobarbital; however such a result might not have been indicated had the rats been subjected to drug administration over a longer interval of time. The data from these experiments did, however, indicate that learning did take place in the experimental rats while they were under the drug. These data suggest that the drug may not have inter-

fered with learning but may have in some way hindered the ability of the rats to utilize what they had learned. Just how the drug acted to cause such a result is not clear. This problem and the problem of possible permanent damage due to the drug offer opportunity for further study.

Experiment III

The significant findings in this experiment seemed to indicate that under conditions of stress phenobarbital significantly reduced visceral reactions accompanying stress. The reduction in visceral reactions might be considered symptomatic of fortification against stress in the drugged rats. Reduction in visceral reactions might also be considered as the cause of more efficient conditioning in drugged rats under stress. In other words, the drugged animals would not be subjected to such a large degree of added stress due to excessive visceral reactions. This would undoubtedly make possible more efficient conditioning for there would be less visceral distraction.

In addition to scored data contained in this experiment, other reactions were observed. In the majority of the control animals flacidity, excitement and vocal reactions were observed not only during the experiment but as soon as the animals were placed into the stress box prior to starting the buzzer or the electric shock. Many of the control animals also showed visceral reactions during this period. Such reactions were almost entire-

ly absent in the experimental animals prior to stimulation and were present to a much lesser degree in them than in the controls during experimental stimulation.

CONCLUSIONS

The data from these experiments seem to warrant the following conclusions:

1. Under normal environmental stimulation and food motivation, phenobarbital reduces performance efficiency in albino rats in maze learning.

2. No evidence was found indicating that phenobarbital reduced maze learning in white rats.

3. Under conditions of stress, phenobarbital caused a reduction in visceral reactions, and nervous reactions such as flacidity, vocal responses, excitement, and visceral reactions occurring previous to stressing stimulation. Under stress conditions phenobarbital also aids in conditioning a response in albino rats.

4. The interference with performance in albino rats due to the drug does not seem to be due to physical disability or interference with motivation when a dosage of .075 grain is administered.

5. No evidence was found indicating that permanent damage to learning ability, memory, or physical disability occurred in the drugged animals. Had the dosage been extended for a greater

length of time than was used in this experiment, such results might not have been obtained.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Dr. O. W. Alm his major instructor, who gave many valuable suggestions throughout the progress of this study and who furnished the apparatus for this investigation. The writer also wishes to acknowledge his indebtedness to Dr. D. F. Showalter who gave him valuable counsel concerning this study.

BIBLIOGRAPHY

(1) Alm, O. W. and C. H. Whitnah.
The relationship between brain lipids and learning ability of albino rats. Journal of Genetic Psychology, 339-403, 1936.

(2) Tatum, A. L.
The present status of the barbiturate problem. Physiological Review, 19:472-481. 1939.

(3) Caspari, Charles.
Phenobarbital, elixor of and soluble. Treatise on Pharmacy, Philadelphia, Lea & Febiger Co. 1930.

(4) Lundy, J. S.
Phenobarbital, sodium and chemistry of. Clinical Anesthesia. Philadelphia. W. B. Saunders Co. 1946.

(5) Torald, Sollmann.
Phenobarbital and the habitual use of the barbiturates. A Manual of Pharmacology. Philadelphia. W. B. Saunders Co. 1942.

(6) Jackson, D. E.
Barbiturates and metrazol. Experimental Pharmacology and Materia Medica. St. Louis. W. B. Saunders Co. 1938.

(7) Adriani, John.
Phenobarbital. Chemistry of Anesthesia. Springfield, Ill. Charles C. Thomas; Publisher. 1946.

APPENDIX

RAW SCORES FOR ANIMAL IN MAZE LEARNING

Experimental Group
(50 trials)

	Animal no.	Errors	Time
Cage A	1	393	596
	2	322	390
	3	336	406
	4	292	418
	5	330	403
	6	490	529
Cage B	1	316	392
	2	346	430
	3	214	347
	4	317	390
	5	536	600
	6	402	530
Cage C	1	360	444
	2	315	429
	3	221	294
	4	250	366
	5	220	317
	6	502	695
Cage D	1	238	447
	2	248	296

Total Errors	6548
Mean Errors	327.40
Total Time	11548 sec.
Mean Time	435.60 sec.
Total Unit Run	15548
Mean Unit Run	777.40
Mean Speed	1.78 U.P.S.

Control Group
(50 trials)

	Animal no.	Errors	Time
Cage A	1	264	396
	2	218	397
	3	257	319
	4	230	571
	5	306	429
	6	290	420
Cage B	1	393	563
	2	50	214
	3	192	442
	4	361	609
	5	316	724
	6	252	336
Cage C	1	308	407
	2	176	314
	3	169	374
	4	240	354
	5	234	302
	6	204	297
Cage D	1	178	350
	2	250	435

Total Errors	4799
Mean Errors	239.95
Total Time	8243 sec.
Mean Time	412.15 sec.
Total Unit Run	13799
Mean Unit Run	689.95
Mean Speed	1.64 U.P.S.

DAY SCORES PER ANIMAL ON MAZE LEARNING

Last 15 Runs Out of 50 Prior to 10 Day Vacation

Cage	Animal no. (Experimental)	Errors	Time	Animal no. (Control)	Errors	Time
A	1	64	124	1	56	96
	2	68	102	2	26	75
	3	60	95	3	56	96
	4	40	85	4	24	58
	5	54	80	5	42	112
	6	114	151	6	28	92
B	1	54	75	1	88	128
	2	54	79	2	0	40
	3	48	88	3	2	52
	4	64	82	4	68	99
	5	130	127	5	71	130
	6	96	107	6	46	66
C	1	88	102	1	38	78
	2	60	112	2	18	58
	3	28	54	3	12	55
	4	38	78	4	46	88
	5	18	58	5	14	54
	6	78	136	6	14	55
D	1	66	114	1	20	65
	2	56	81	2	30	30

Total Errors	1279	Total Errors	653
Mean Errors	65.95	Mean Errors	32.65
Total Time	1905	Total Time	1575
Mean Time	95.25	Mean Time	78.75

RAW SCORES FOR ANIMAL NO MAZE MASTERED

Last 15 Runs Out of 25 After 10 Day Vacation

Cage	Animal no. (Experimental)	Errors	Time	Animal no. (Control)	Errors	Time
A	1	38	69	1	44	61
	2	22	64	2	16	53
	3	34	68	3	6	46
	4	13	69	4	38	57
	5	30	69	5	64	100
	6	34	69	6	23	72
B	1	66	85	1	36	87
	2	28	64	2	0	34
	3	14	51	3	0	40
	4	28	65	4	60	86
	5	90	120	5	40	83
	6	72	102	6	26	61
C	1	30	71	1	14	50
	2	34	62	2	30	58
	3	28	66	3	36	73
	4	26	63	4	46	62
	5	24	56	5	10	49
	6	60	87	6	20	51
D	1	46	94	1	14	50
	2	44	78	2	16	51

Total Errors	767	Total Errors	583
Mean Errors	38.35	Mean Errors	29.40
Total Time	1467	Total Time	1239
Mean Time	73.35	Mean Time	61.95

Raw Scores per Animal Each in Experiment
With Stress Box (100 Signals)

Experimental Group

Cage	Animal no.	Total Defecation Pellets	Total Urinations	Signal Runs	Exposures
A	1	27	13	34	86
	2	12	13	20	80
	3	9	4	59	41
	4	15	8	14	86
	5	8	5	27	73
	6	11	5	31	71
B	1	23	11	14	87
	2	17	12	38	63
	3	22	9	9	92
	4	19	11	28	74
	5	16	12	19	81
	6	17	11	23	77
C	1	28	10	33	68
	2	15	19	61	40
	3	22	11	30	70
	4	8	10	25	75
	5	9	5	38	63
	6	12	11	30	63
D	1	19	9	26	73
	2	19	9	19	82
Totals		326	180	506	1430
Means		16.30	9.4	29.30	71.50

Weight Loss For Experimental Group

Cage	Animal no.	Grams	Cage	Animal no.	Grams
A	1	-15	C	1	0
	2	-10		2	-12
	3	-42		3	-12
	4	+ 7		4	+10
	5	-21		5	-20
	6	00		6	-19
B	1	- 4	D	1	- 4
	2	00		2	- 9
	3	-13			
	4	+ 6	Total		-1700 Gms
	5	- 3	Mean Wt. Loss		- 8.5 Gms
	6	00			

Raw Scores per Animal Made in Experiment
With Stress Box (100 Signals)

Control Group

Cage	Animal no.	Total Defecation Pellets	Total Urinations	Signal Runs	Exposures
A	1	35	13	8	94
	2	34	13	23	71
	3	35	12	17	83
	4	24	11	6	94
	5	30	13	17	85
	6	41	12	3	91
B	1	36	11	7	93
	2	44	12	22	79
	3	49	12	4	97
	4	33	12	17	85
	5	34	11	16	84
	6	42	13	12	88
C	1	40	13	3	97
	2	44	12	20	80
	3	31	10	16	84
	4	39	11	3	98
	5	32	10	26	74
	6	35	10	16	83
D	1	31	12	7	93
	2	35	10	4	96
Totals		725	233	252	1754
Means		36.25	11.65	12.9	87.70

Weight Loss for Control Group

Cage	Animal no.	Grams	Cage	Animal no.	Grams
A	1	-30	C	1	-17
	2	-11		2	-14
	3	- 4		3	-19
	4	+ 6		4	-12
	5	-10		5	-19
	6	-34		6	-13
B	1	+ 2	D	1	-13
	2	+ 6		2	-25
	3	- 6			
	4	-12	Total		-254
	5	-11	Mean Wt. Loss		12.7
	6	-18			

Statistical Formulae Used

1. The Standard Error of the Difference between the Means

$$\sigma_{\bar{X}-\bar{X}} = \sqrt{\left(\frac{\Sigma x_1^2 + \Sigma x_2^2}{N + N-2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$$

2. Significance Ratio

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sigma_{\bar{X}_1-\bar{X}_2}}$$